SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS WR-061-94
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT This document has been approved for public release and sale; its distribution is unlimited.
2b. [illegible] AD-A286 622	
4. PE	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION DIVISION OF BIOCHEMISTRY	6b. OFFICE SYMBOL (If applicable) SGRD-UWG
6c. ADDRESS (City, State, and ZIP Code) WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, DC 20307-5100	7a. NAME OF MONITORING ORGANIZATION 7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND	8b. OFFICE SYMBOL (If applicable)
8c. ADDRESS (City, State, and ZIP Code) FORT DETRICK, FREDERICK, MD 21701 WALTER REED ARMY INSTITUTE OF RESEARCH WASHINGTON, DC 20307-5100	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

DTIC ELECTE S NOV 2 3 1994 D F

10. SOURCE OF FUNDING NUMBERS			
PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
CYTOTOXIC T LYMPHOCYTES INDUCED BY LIPOSOMAL ANTIGENS: MECHANISMS OF IMMUNOLOGICAL PRESENTATION

12. PERSONAL AUTHOR(S)
CARL R. ALVING and NABILA M. WASSEF

13a. TYPE OF REPORT	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT

16. SUPPLEMENTARY NOTATION

DTIC QUALITY INSPECTED 5

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
			LIPOSOMES, ANTIGEN PRESENTATION, CYTOTOXIC T LYMPHOCYTES

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

It is known that liposomes can deliver encapsulated substances, including drugs and antigens, to lysosomes in macrophages. Because of this it has been assumed that although liposomes might be useful for induction of humoral (class II) immunity, they would not be capable of cytoplasmic delivery of antigen for introduction into the class I pathway leading to induction of cytotoxic T lymphocytes (CTLs). However, experiments conducted by numerous laboratories, including our own, have demonstrated the ability to induce CTLs either *in vitro* with cultured cells incubated with liposome-associated antigen, or *in vivo* after immunization of mice or monkeys with liposomes containing associated antigen. Using a monoclonal antibody that recognizes repeating sequences of tetrapeptide epitopes derived from the circumsporozoite protein of *Plasmodium falciparum*, it has been shown by immunogold electron microscopy that liposomal antigenic epitopes can actually spill from endosomes into the cytoplasm of cultured macrophages. On the basis of this observation, a theoretical intracellular pathway is proposed whereby liposomal antigen is processed by macrophages through a cytoplasmic process that results in delivery of antigenic epitopes to the Golgi apparatus and the endoplasmic reticulum. The liposomal antigenic epitopes would then have the opportunity to associate with class I MHC molecules and undergo vesicular transport to the surface of the cells for presentation and induction of CTLs.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 10, Supplement 2, 1994
Mary Ann Liebert, Inc., Publishers

Novel Vaccines and Adjuvants: Mechanisms of Action

Cytotoxic T Lymphocytes Induced by Liposomal Antigens: Mechanisms of Immunological Presentation

CARL R. ALVING and NABILA M. WASSEF

ABSTRACT

It is known that liposomes can deliver encapsulated substances, including drugs and antigens, to lysosomes in macrophages. Because of this it has been assumed that although liposomes might be useful for induction of humoral (class II) immunity, they would not be capable of cytoplasmic delivery of antigen for introduction into the class I pathway leading to induction of cytotoxic T lymphocytes (CTLs). However, experiments conducted by numerous laboratories, including our own, have demonstrated the ability to induce CTLs either *in vitro* with cultured cells incubated with liposome-associated antigen, or *in vivo* after immunization of mice or monkeys with liposomes containing associated antigen. Using a monoclonal antibody that recognizes repeating sequences of tetrapeptide epitopes derived from the circumsporozoite protein of *Plasmodium falciparum*, it has been shown by immunogold electron microscopy that liposomal antigenic epitopes can actually spill from endosomes into the cytoplasm of cultured macrophages. On the basis of this observation, a theoretical intracellular pathway is proposed whereby liposomal antigen is processed by macrophages through a cytoplasmic process that results in delivery of antigenic epitopes to the Golgi apparatus and the endoplasmic reticulum. The liposomal antigenic epitopes would then have the opportunity to associate with class I MHC molecules and undergo vesicular transport to the surface of the cells for presentation and induction of CTLs.

INTRODUCTION

IN THE SEARCH for useful modern adjuvants it has become evident that the immunostimulating mechanisms of adjuvants and adjuvant formulations frequently are complex and are often poorly understood. Among many mechanisms that have been identified for different immunostimulating substances are the following: depot effect for slow release of antigen, binding or adsorption of antigen, targeting of antigen to antigen-presenting cells (APCs), reconstitution of antigen and presentation of T and B epitopes, recruitment of immune cells, activation of complement, induction of cytokine production, and modulation of MHC class I or class II expression.[1–3]

Liposome research has been a major beneficiary of a resurgence of interest in vaccine adjuvants (reviewed in Refs. 1 and 4–7). Although liposomes were originally developed as models of efferent mechanisms exhibited by the immune response, it has now become evident that antigens that are presented or reconstituted in liposomes can provide desirable properties that promote effective humoral and cellular immune responses in many vaccines. Liposomes have been proposed as vehicles for vaccines against parasitic and viral illnesses. Experimental vaccines against malaria, HIV, hepatitis A, and influenza virus have been shown to be safe and highly immunogenic in several human trials.[8–13]

INDUCTION OF CYTOTOXIC T LYMPHOCYTES

Humoral and cellular pathways are both major elements in the generation of immune responses. Induction of cytotoxic T lymphocytes (CTLs) has been proposed as a useful strategy for developing vaccines against intracellular antigens, such as viral, parasitic, or tumor antigens.[14] Exogenous antigens, such as synthetic soluble peptides or proteins, or for that matter any extracellular antigen, usually must enter the cytoplasm of an APC in order to participate in the processing pathway leading to pre-

Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

sentation with MHC class I molecules to induce CD8+ CTLs. This principle was illustrated by a well-known study in which purified antigen was directly introduced into the cytoplasm of cells by osmotic lysis of pinosomes.[15] Studies have also indicated that macrophages can serve as APCs for generation of CTLs.[16] Regardless of the cell type involved, the likely intracellular pathway by which cytoplasmic antigen normally gains access to class I MHC molecules for presentation involves partial degradation of antigen by "proteasomes,"[17] delivery of immunogenic peptides to the endoplasmic reticulum via a "peptide transporter" mechanism, complexing of the peptide with MHC class I molecules, transport of the complex to the Golgi, and subsequent vesicular transport to the surface of the cell for presentation to T cells.[18,19]

MECHANISMS OF CYTOTOXIC T LYMPHOCYTE INDUCTION BY LIPOSOMES

Numerous reports have now described class I presentation and induction of CTLs by liposomal antigens. These have included both *in vitro* studies.[20–26] and *in vivo* studies[27–36] with many different antigens (Table 1).

Cytoplasmic delivery of antigen can be facilitated in cultured cells by so-called "pH-sensitive" or "acid-sensitive" liposomes.[25,26,37] Although "acid-insensitive" liposomes were initially thought to be excluded from the cytoplasm of macrophages,[25,26,37] subsequent research demonstrated that cytoplasmic delivery and class I presentation also occurred with "acid-insensitive" liposomes.[24] Numerous *in vivo* studies have shown that "acid-insensitive" liposomes can readily induce CTLs.[27–36] Cytotoxic T lymphocyte induction was also facilitated by utilizing liposomes containing a fusion protein in order to introduce liposomal antigen directly into cells.[35] In an *in vivo* murine model, CTLs were even generated by encapsulation of an extremely small (15-amino acid) unconjugated peptide in liposomes containing lipid A.[38]

The ability of liposomes to deliver antigens to macrophages as antigen-presenting cells has been presumed to be the underlying mechanism that results in potent humoral immune responses to the liposomes.[4,6] The immune response can also be

TABLE 1. INDUCTION OF CYTOTOXIC T LYMPHOCYTES BY LIPOSOME-ENCAPSULATED ANTIGEN

Antigen	*Liposome composition*	*Ref.*
In vitro studies		
MHC antigens (H-2 in mice)	Egg PC/CHOL (70:30, w/w)	Hale[20]
MHC antigens (H-2 in mice)	Egg PC/CHOL (70:30, w/w)	Hale and McGee[21]
Human colon tumor antigens (LS174T colon tumor cells)	PC/CHOL/PA (7:2:1)	Raphael and Tom[22,23]
Ovalbumin Murine hemoglobin Bovine ribonuclease Hen egg lysozyme	DOPC/DOPS (4:1) and DOPE-PHC (4:1)	Harding *et al.*[24]
Ovalbumin	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Reddy *et al.*[25]
Ovalbumin	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Zhou *et al.*[26]
In vivo studies		
Hemagglutinin, neuraminidase	MDP, and MDP/CHOL (1:1, w/w)	Nerome *et al.*[27]
Ovalbumin, β-galactosidase	DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3)	Reddy *et al.*[28]
Ovalbumin	DOPE/DOSG,DOPE/DPSG, DOPE/CHEMS, DOPC/DOPS, (all 4:1), ± 50 μg of lipid A DPPC/DPPG/CHOL (9:1:8) ± 50 μg of lipid A	Collins *et al.*[29]
Ovalbumin	PC/lysoPC/CHOL (6.9:0.1:3, neutral), PC/lysoPC/SA/CHOL (6.9:0.1:1:2, positive), PC/lysoPC/DCP/CHOL (6.9:0.1:1:2, negative)	Lopes and Chain[30]
Multiple antigen peptide system (MAPS) from gp120 of HIV-1 (B2M-P3C)	Egg PC/CHOL/SA (7.5:1:0.25, w/w)	Defoort *et al.*[31]
Glycoprotein B from HSV	Cationic lipids (dioleoyloxypropyl-trimethyl-ammonium methyl sulfate, DOTAP)	Walker *et al.*[32]
Ovalbumin	Commercially available DOTAP	Chen *et al.*[33]
Repeatless *Plasmodium falciparum* CS protein	DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.026)	White *et al.*[34]
SIV Gag protein-derived peptide (p11C)	PS/CHOL (9:1) envelope glycoproteins and lipids of Sendi virus	Miller *et al.*[35]
SIV Gag protein-derived peptide (M90-07A)	DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.1)	Yasutomi *et al.*[36]

CHEMS, Cholesterol hemisuccinate; CHOL, cholesterol; CS, circumsporozoite; DCP, dicetyl phosphate; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DOPC, dioleoyl phosphatidylcholine; DOPE, dioleoyl phosphatidylethanolamine; DOPS, dioleoyl phosphatidylserine; DOSG, 1,2-dioleoyl-sn-3-succinylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DPPG, dipalmitoyl phosphatidylglycerol; DPSG, dipalmitoyl succinylglycerol; lysoPC, lysophosphatidylcholine; MDP, muramyl dipeptide; MHC, major histocompatibility antigen gene complex; PA, phosphatidic acid; PC, phosphatidylcholine; PHC, palmitoyl homocysteine; PS, phosphatidylserine; SA, stearylamine.

enhanced by the presence of lipid A, the endotoxic moiety of bacterial lipopolysaccharide, as a simultaneous constituent that serves as an adjuvant in the liposomes.[39] In accordance with this, we have demonstrated that liposomal lipid A can serve as a stimulant for increased specific presentation of phagocytosed liposomal antigen by macrophages.[40]

Proposed intracellular processing pathway for induction of cytotoxic T lymphocyte by liposomes

The same liposomes that were used for presentation studies in macrophages have also been employed for induction of CTLs with an encapsulated antigen containing a CTL epitope.[34] The ability of antigen contained within liposomes to enter the cytoplasmic class I pathway for induction of CTLs was anticipated by an immunogold electron microscopy study that demonstrated that liposomal antigen was disgorged in large amounts into the cytoplasm of macrophages (Fig. 1).[41] On the basis of this we propose that liposomes, or lipid–peptide complexes, that escape into the cytoplasm of macrophages can gain access to class I MHC molecules in the Golgi apparatus (Fig. 2).

Investigations performed in the laboratories of Pagano[42–44] and Ohnishi[45] have suggested that liposomes in the cytoplasm can gain direct access to the Golgi apparatus through an ATP-dependent fusion phenomenon that is mediated by a Golgi-associated protein that recognizes the liposomal lipids. We propose that the liposomes containing antigen, or partially degraded lipid peptide complexes, enter the cytoplasm of macrophages (Fig. 2). The complexes are then either taken up directly by the Golgi via the ATP-dependent fusion phenomenon described by Pagano and Ohnishi, or alternatively liposomal peptide in the cytoplasm is transported to the endoplasmic reticulum by the cytoplasmic peptide transporter (Fig. 2). The liposomal peptide would thereby gain access to the classic intracellular pathway for class I presentation of cytoplasmic antigen.

The cell biology mechanism that we propose, as shown in Fig. 2, is amenable to experimental testing. In pursuing this, experiments are currently underway, using fluorescent-labeled liposomes and antigen, to determine whether liposomal antigenic epitopes that escape from low-pH vacuoles into the cytoplasm of macrophages are actually directly delivered to the Golgi apparatus or to the endoplasmic reticulum. If this mechanism proves to be widely applicable to different antigens it could provide a broad theoretical basis for utilization of liposomes as carriers of antigens for induction of CTLs.

FIG. 1. Immunogold electron microscopy of cultured bone marrow-derived macrophages after phagocytosis of liposomes containing malaria antigen. The macrophages were fixed after incubation with the liposomes. The malaria antigen was detected by an antigen-specific monoclonal antibody (Pf 1B2.2), followed by treatment with a gold-labeled second antibody. Cytoplasmic antigen is indicated by four arrows. (From Verma *et al.*[41])

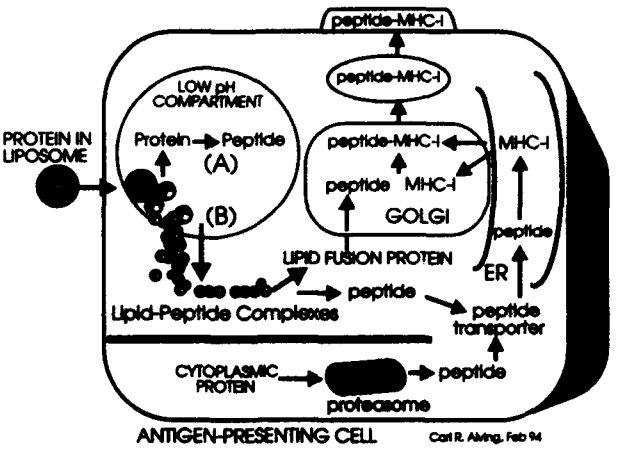

FIG. 2. Schematic representation of proposed presentation of liposomal antigen in MHC class I pathway.

REFERENCES

1. Alving CR: Immunologic aspects of liposomes: Presentation and processing of liposomal protein and phospholipid antigens. Biochim Biophys Acta 1992;1113:307–322.
2. Alving CR, Glass M, and Detrick B: Summary: Adjuvants/clinical working group. AIDS Res Hum Retroviruses 1992;8:1427–1430.
3. Alving CR, Detrick B, Richards RL, Lewis MG, Shafferman A, and Eddy GA: Novel adjuvant strategies for experimental malaria and AIDS vaccines. Ann NY Acad Sci 1993;690:265–275.
4. Su D and van Rooijen N: The role of macrophages in the immunoadjuvant action of liposomes: Effects of elimination of splenic macrophages on the immune response against intravenously injected liposome-associated albumin antigen. Immunology 1989;66:466–470.
5. Gregoriadis G: Immunological adjuvants: A role for liposomes. Immunol Today 1990;11:89–97.
6. Alving CR: Liposomes as carriers of antigens and adjuvants. J Immunol Methods 1991;140:1–13.
7. Phillips NC: Liposomal carriers for the treatment of acquired immune deficiency syndromes. Bull Inst Pasteur 1992;90:205–230.
8. Fries LF, Gordon DM, Richards RL, Egan JE, Hollingdale MR, Gross M, Silverman C, and Alving CR: Liposomal malaria vaccine in humans: A safe and potent adjuvant strategy. Proc Natl Acad Sci USA 1992;89:358–362.
9. Glück R: Immunopotentiating reconstituted influenza virosomes (IRIVs) and other adjuvants for improved presentation of small antigens. Vaccine 1992;10:915–919.
10. Glück R, Mischler R, Brantschen S, Just M, Althaus B, and Cryz SJ

Jr: Immunopotentiating reconstituted influenza virus virosome vaccine delivery system for immunization against hepatitis A. J Clin Invest 1992;90:2491–2495.
11. Just M, Berger R, Drechsler H, Brantschen S, and Glück R: A single vaccination with an inactivated hepatitis A liposome vaccine induces protective antibodies after only two weeks. Vaccine 1992;10:737–739.
12. Kaji M, Kaji Y, Kaji M, Ohkuma K, Honda T, Oka T, Sakoh M, Nakamura S, Kurachi K, and Sentoku M: Phase 1 clinical tests of influenza MDP-virosome vaccine (KD-5382). Vaccine 1992; 10:663–667.
13. Alving CR: Liposomes as vehicles for vaccines. In: *Handbook of Natural Toxins*, Vol. 8: *Microbiol Toxins*. Iglewski B, Vaughan M, Tu AT, and Moss J (Eds.). Marcel Dekker, New York, 1994 (in press).
14. Rouse BT, Norley S, and Martin S: Antiviral cytotoxic T lymphocyte induction and vaccination. Rev Infect Dis 1988;10:16–32.
15. Moore MW, Carbone FR, and Bevan MJ: Introduction of soluble protein into the class I pathway of antigen processing and presentation. Cell 1988;54:777–785.
16. Debrick JE, Campbell PA, and Staerz UD: Macrophages as accessory cells for class I MHC-restricted immune responses. J Immunol 1991;147:2846–2851.
17. Arrigo A, Tanaka K, Goldberb AL, and Welch WJ: Identity of the 19S "prosome" particle with the large multifunctional protease complex of mammalian cells (the proteasome). Nature (London) 1988;331:192–194.
18. Yewdell JW and Bennick JR: The binary logic of antigen processing and presentation to T cells. Cell 1990;62:203–206.
19. Driscoll J and Finley D: A controlled breakdown: Antigen processing and the turnover of viral proteins. Cell 1992;68:823–825.
20. Hale AH: H-2 antigens incorporated into phospholipid vesicles elicit specific allogeneic cytotoxic T lymphocytes. Cell Immunol 1980;55:328–341.
21. Hale AH and McGee MP: A study of the inability of subcellular fractions to elicit primary anti-H-2 cytotoxic T lymphocytes. Cell Immunol 1981;58:277–285.
22. Raphael L and Tom BH: In vitro induction of primary and secondary xenoimmune responses by liposomes containing human colon tumor cell antigens. Cell Immunol 1982;71:224–240.
23. Raphael L and Tom BH: Liposome facilitated xenogeneic approach for studying human colon cancer immunity: Carrier and adjuvant effect of liposomes. Clin Exp Immunol 1984;55:1–13.
24. Harding CV, Collins DS, Kanagawa O, and Unanue ER: Liposome-encapsulated antigens engender lysosomal processing for class II MHC presentation and cytosolic processing for class I presentation. J Immunol 1991;147:2860–2863.
25. Reddy R, Zhou F, Huang L, Carbone F, Bevan M, and Rouse BT: pH sensitive liposomes provide an efficient means of sensitizing target cells to class I restricted CTL recognition of a soluble protein. J Immunol Methods 1991;141:157–163.
26. Zhou F, Rouse BT, and Huang L: An improved method of loading pH-sensitive liposomes with soluble proteins for class I restricted antigen presentation. J Immunol Methods 1991;145:143–152.
27. Nerome K, Yoshioka Y, Ishida M, Okuma K, Oka T, Kataoka T, Inoue A, and Oya A: Development of a new type of influenza subunit vaccine made by muramyldipeptide-liposome: Enhancement of humoral and cellular immune responses. Vaccine 1990;8: 503–509.
28. Reddy R, Zhou F, Nair S, Huang L, and Rouse BT: In vivo cytotoxic T lymphocyte induction with soluble proteins administered in liposomes. J Immunol 1992;148:1585–1589.
29. Collins DS, Findlay K, and Harding CV: Processing of exogenous liposome-encapsulated antigens in vivo generates class I MHC-restricted T cell responses. J Immunol 1992;148:3336–3341.
30. Lopes LM and Chain BM: Liposome-mediated delivery stimulates a class I-restricted cytotoxic T cell response to soluble antigen. Eur J Immunol 1992;22:287–290.
31. Defoort J-P, Nardelli B, Huang W, and Tam JP: A rational design of synthetic peptide vaccine with a built-in adjuvant. Int J Peptide Protein Res 1992;40:214–221.
32. Walker C, Selby M, Erickson A, Cataldo D, Valensi J, and Van Nest G: Cationic lipids direct a viral glycoprotein into the class I major histocompatibility complex antigen-presentation pathway. Proc Natl Acad Sci USA 1992;89:7915–7918.
33. Chen W, Carbone FR, and McCluskey J: Electroporation and commercial liposomes efficiently deliver soluble protein into the MHC class I presentation pathway. J Immunol Methods 1993;160:49–57.
34. White K, Krzych U, Gordon DM, Porter TG, Richards RL, Alving CR, Deal CD, Hollingdale M, Silverman C, Sylvester DR, Ballou WR, and Gross M: Induction of cytolytic and antibody responses using *P. falciparum* repeatless circumsporozoite protein encapsulated in liposomes. Vaccine 1993;11:1341–1346.
35. Miller MD, Gould-Fogerite S, Shen L, Woods RM, Koenig S, Mannino RJ, and Letvin NL: Vaccination of rhesus monkeys with synthetic peptide in a fusogenic proteoliposome elicits simian immunodeficiency virus-specific CD-8+ cytotoxic T lymphocytes. J Exp Med 1992;176:1739–1744.
36. Yasutomi Y, Alving CR, Wassef NM, Conard P, Conley AJ, Emini EA, Madsen J, Woods R, Koenig S, and Letvin NL: Combined modality immunization for elicitation of SIV_{mac} gag-specific CTL. Vaccine 1994 (in press).
37. Harding CV, Collins DS, Slot JW, Geuze HJ, and Unanue E: Liposome-encapsulated antigens are processed in lysosomes, recycled, and presented to T cells. Cell 1991;64:393–401.
38. White WI, Cassatt DR, Madsen J, Burke SJ, Woods RM, Wassef NM, Alving CR, and Koenig S: Induction of both antibody and cytotoxic T lymphocyte responses to a liposome-associated HIV-1 peptide (submitted for publication).
39. Alving CR: Lipopolysaccharide, lipid A, and liposomes containing lipid A as immunologic adjuvants. Immunobiology 1993;187: 430–446.
40. Verma JN, Rao M, Amselem S, Krzych U, Alving CR, Green SJ, and Wassef NM: Adjuvant effects of liposomes containing lipid A: Enhancement of liposomal antigen presentation and recruitment of macrophages. Infect Immun 1992;60:2438–2444.
41. Verma JN, Wassef NM, Wirtz RA, Atkinson CT, Aikawa M, Loomis LD, and Alving CR: Phagocytosis of liposomes by macrophages: Intracellular fate of liposomal malaria antigen. Biochim Biophys Acta 1991;1066:229–238.
42. Kobayashi T and Pagano RE: ATP-dependent fusion of liposomes with the Golgi apparatus of perforated cells. Cell 1988;55: 797–805.
43. Pagano RE: The Golgi apparatus: Insights from lipid biochemistry. Biochem Soc Trans 1990;18:361–366.
44. Pagano RE: Lipid traffic in eukaryotic cells: Mechanisms for intracellular transport and organelle-specific enrichment of lipids. Curr Opin Cell Biol 1990;2:652–663.
45. Kagiwada S, Murata M, Hishida R, Tagaya M, Yamashina S, and Ohnishi S: *In vitro* fusion of rabbit liver Golgi membranes with liposomes. J Biol Chem 1993;268:1430–1435.

Address reprint requests to:
Carl R. Alving
Department of Membrane Biochemistry
Walter Reed Army Institute of Research
Washington, D.C. 20307-5100